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(MMPs) which degrade the cartilage matrix. Of the cytokines implicated in the pathophysiology of OA, IL-1 has attracted the most interest as a target for disease modification and IL-1 inhibition has been reported to be beneficial in several animal models of OA. In considering potential barriers that may limit the use of biological therapeutics in treating cartilage-associated diseases, we asked whether two IL-1 receptor antagonists were capable of penetrating cartilage and reaching the IL-1 receptor on chondrocytes. In the present study we evaluate the efficacy of IL-1ra (Kineret; ~17 kDa) and a monoclonal IL-1R antibody (6F3; ~150 kDa)) as antagonists of IL-1 in human chondrocytes and cartilage explants from non-arthritic donors.

Methods: Human primary chondrocytes and cartilage explants (3-mm diameter) were obtained from Articular Engineering (Chicago, IL). Chondrocytes and cartilage explants were incubated with Kineret or 6F3 for 30 min prior to stimulation with 1 ng/ml IL-1β. MMP-13 levels in the conditioned media were measured using an MMP-13 Fluorokine Assay (R&D Systems). Detection of endogenous antibodies in the joints of rat, wild-type mice or mice expressing human immunoglobulins was assessed by immunohistochemistry using secondary anti-rat, anti-mouse or anti-human antibodies.

Results: In human primary chondrocyte cultures, both Kineret and 6F3 were potent inhibitors of IL-1 induced MMP-13 production. Whereas Kineret retained its potency in cartilage explants, the potency of 6F3 was dramatically diminished in cartilage explants, suggesting that the larger molecule was impaired from reaching its target by the extracellular matrix. To further address whether antibodies penetrate cartilage, the detection of endogenous antibodies in the joints of rat, wild-type mice or mice expressing human immunoglobulins was assessed by immunohistochemistry using secondary anti-rat, anti-mouse or anti-human antibodies. Endogenous rat, mouse or human antibodies were not present in either articular or growth plate cartilage. In contrast, saturating levels of endogenous antibody was detected in the adjacent fibrocartilage and soft tissues including the synovium, ligaments, the joint capsule and stroma surrounding bone marrow blood vessels.

Conclusions: Collectively, these data demonstrate that antibodies do not normally penetrate either articular or growth plate cartilage. As such, antibodies do not represent a viable therapeutic modality for targeting chondrocyte-mediated processes. Smaller alternatives such as Fab′ fragments and non-antibody scaffolds may offer advantages over antibodies for achieving cartilage penetration.

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TROPHIC EFFECTS OF MESENCHYMAL STEM CELLS INCREASE CHONDROCYTE PROLIFERATION AND MATRIX FORMATION

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Purpose: Previous studies have shown that co-culture of primary chondrocytes with various sources of multipotent cells results in a higher relative amount of cartilage matrix formation than cultures containing only chondrocytes. The aim of this study is to investigate the mechanism underlying this observation.

Methods: *Cell culture*: Human primary chondrocytes (PC) were isolated from cartilage biopsies of patients undergoing total knee replacement after collagenase II digestion. Human mesenchymal stem cells were isolated from bone marrow aspirates. The use of all human biopsies in this study was approved by a local Medical Ethical Committee.

Histology: Cell pellets were fixed and embedded in paraffin using routine procedures. Sections were cut and stained for sulfated glycosaminoglycans (GAG) with alcian blue combined with counterstaining of nuclear fast red.

GAG and DNA assay: Cell pellets were were digested with proteinase K to release GAGs. Then GAG content was spectrophotometrically determined with 1,9-dimethylmethylene blue chloride using an ELISA reader. Cell number was determined by quantification of total DNA using a CyQuant DNA Kit.

EdU staining and quantification: EdU (5-ethynyl-2′-deoxyuridine) was added to the culture media to label proliferating cells. Pellets were cryosectioned and stained for EdU with Click-iT® EdU Imaging Kit. Nuclei were counterstained with Hoechst 33342. Images were taken and analyzed with ImageJ.

Short Tandem Repeats (STR) analysis. STR analyses of genomic DNA samples were performed with PowerPlex 16 System. The amount of DNA present for each donor was calculated from the areas of the electropherogram for each locus of MSCs' or PCs' specific alleles and the ratio of MSCs and PCs was determined.

Results: Cocultures increase GAG formation. Histology and GAG quantification were performed to evaluate cartilage formation. Alcian blue staining indicated the presence of GAG in all experimental groups. In the positively stained areas, cells showed typical chondrocyte morphology and embedding in lacunae. GAG quantification showed a trend of increasing total GAG with increasing initial percentage of PC. When total GAG content was normalized to the initial percentage of PC, co-culture pellets showed significantly higher GAG content than monocultures.

Cocultures increase chondrocytes proliferation. Cell proliferation was then examined by using EdU incorporation. PCs were labeled with CM-DiI to distinguish them from MSCs. At day 2, EdU positive cells were predominantly found at the periphery of the pellets where red labeled PCs resided. The percentage of EdU positive MSCs or PC was determined. PCs in cocultures proliferate at higher rate than PC in monocutlure.

Ratio of MSCs decreases after coculture. To determine the ratio of MSC and PC after coculture STR loci in different donors were analyzed. The results indicated that the proportion of MSCs decreased significantly after 4 weeks cocultures.

Conclusions: In pellet cocultures, MSC act as trophic mediators stimulating cartilage matrix formation by promoting proliferation of primary chondrocytes rather than differentiating into chondrocytes.

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THROMBIN AND PLASMIN ARE ABLE TO INDUCE PROTEOGLYCAN RELEASE IN A PAR-INDEPENDENT MANNER IN HUMAN CARTILAGE

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Purpose: Osteoarthritis (OA) and Rheumatoid Arthrisis (RA) are characterized by degradation of the cartilage. Proteases of the coagulation cascade and the fibrinolytic system are elevated in both plasma and synovial fluid of OA and RA patients. Cross-talking between coagulation and inflammation is mediated by protease-activated-receptors (PARs), a cell membrane receptor family. These receptors are transmembrane G protein-coupled receptors that are activated through cleavage by serine proteases, such as thrombin and plasmin. PARs are expressed at increased levels in human OA and RA synovium and cartilage. Activation of these PARs results in the induction of catabolic and proinflammatory pathways. Our aim was to study whether thrombin, plasmin and PAR agonists induce cartilage damage in human healthy and OA cartilage. Furthermore, we wanted to investigate if thrombin and plasmin induce cartilage damage in a PAR-dependent manner.

Methods: Healthy full-thickness human articular cartilage tissue was obtained postmortem within 24 hours after death of the donor. Full-thickness OA human articular cartilage tissue was obtained during total knee surgery. Slices of cartilage were cut aseptically from the articular surface and kept in phosphate-buffered saline. Within 1 hour of dissection the slices were cut into square pieces, weighed aseptically (range, 5.0 to 15.0 mg) and each sample was individually put into culture. Cartilage was pre-cultured for two days in medium supplemented with serum. Subsequently, the cartilage was cultured in serum-free medium. After 24h the serum-free medium was refreshed and cartilage was cultured for 4 days in serum-free medium in the presence of different concentrations thrombin (10, 30 or 100nM), plasmin (1, 3, 10, 30 or 100nM), PAR1 agonist TFLLR-NH_2(100 μM), PAR2 agonist SLIGKV (100 μM), with or without co-culture with PAR1 antagonist SCH79797 (100 μM) or PAR2 antagonist FSLLRY-NH_2 (100 μM). Cartilage matrix turnover, in terms of proteoglycan synthesis, -release, and -content, was determined at day 4.

Results: Thrombin and plasmin increased proteoglycan release in both healthy cartilage and osteoarthritis cartilage in a dose-dependent manner (471% for thrombin at 100nM; 744% for plasmin at 100nM; both $p < 0.05$). Proteoglycan synthesis and content were not significantly changed by thrombin or plasmin. Thrombin-induced and plasmin-induced proteoglycan release were not affected by co-culture with either PAR1 antagonist SCH79797, or PAR2 antagonist FSLLRY-NH_2. Also the direct stimulation with PAR1 agonist TFLLR-NH_2, or PAR2 agonist SLIGKV failed to induce proteoglycan release.

Conclusions: Both thrombin and plasmin were able to induce proteoglycan release in a dose-dependent manner. PAR1 and PAR2 antagonists were not able to inhibit the thrombin- and plasmin induced proteoglycan release. Furthermore, the PAR agonists TFLLR-NH_2 and SLIGKV failed to induce proteoglycan release. This suggests that both thrombin and plasmin induce proteoglycan release and that both are able to damage the human cartilage in a PAR-independent manner.

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STUDY OF THE ASSOCIATION BETWEEN BONE SIALOPROTEIN, HYPERTROPHIC DIFFERENTIATION OF CHONDROCYTES AND CARTILAGE LESIONS IN OSTEOARTHRITIC CARTILAGE

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Purpose: Chondrocyte hypertrophy is commonly observed in OA cartilage, associated with matrix mineralization and vascularization. In previous work, we demonstrated that hypertrophic differentiation of chondrocytes is initiated by serum-enriched medium in long-term culture in alginate beads suggesting a role played by blood supply in the hypertrophic differentiation of chondrocytes in OA.
This study aims to investigate the production of Bone SialoProtein (BSP), an angiogenesis enhancer known to promote endothelial cell attachment and migration, during hypertrophic differentiation of OA chondrocytes and its expression in OA cartilage according to the severity of the lesions by immunohistochemistry. This work was supported by an OARSI scholarship grant.
Methods: Articular OA chondrocytes were cultured for 28 days in alginate beads in culture medium containing 2% Ultroser G (UG) or 10% Fetal Bovine Serum (FBS). DNA was quantified by fluorimetry. The expression of BSP and hypertrophic differentiation marker COL10A1 was evaluated by RT-PCR. Alkaline phosphatase (AP) activity and 5′phosphodiesterase activity of NTPPPH were quantified by specific enzymatic methods. Western Blot analysis was performed from chondrocyte protein extracts with anti-BSP antibody (LFMb-24).
Human bone and cartilage samples from 9 post-mortem (PM) individuals and 24 patients undergoing total knee joint replacement for OA (TKR) were formalin-fixed, EDTA-decalcified and wax embedded. Five μm tissue sections were cut and stained. Macroscopic chondropathy score described previously by Walsh et al. and the modified Mankin score were respectively used to establish macroscopic and microscopic chondropathy scores for individual knees. BSP was immunolocalized in cartilage using anti-BSP monoclonal antibody (LFMb-25) and anti-BSP polyclonal antibody (ab52128) to confirm the specificity of staining. A scoring system was established according to the location of the stained cells in cartilage (0: no staining – 1: staining in the superficial layer – 2: staining in the middle layer – 3: staining in the deep layer). All histological scoring was undertaken twice blinded to patient group, using a Zeiss Axioscop-50 microscope.
Data were analyzed using GraphPad Prism software, version 5. Associations between variables were reported as Spearman's rank correlation coefficients (r).
Results: In alginate beads, chondrocytes cultured in serum-supplemented medium underwent a hypertrophic differentiation process characterized by the increased expression of hypertrophic differentiation markers. In the same manner, the expression of BSP increased in FBS with long-term culture and was associated with markers of chondrocytes hypertrophy; COL10A1 ($r = 0.67$; $p = 0.005$), AP ($r = 0.8$; $p = 0.0002$) and NTPPPH ($r = 0.68$; $p = 0.004$). After 21 days, BSP was also detected in protein extracts of chondrocytes cultured in serum but not in UG.
BSP-immunoreactive chondrocytes were localized at increased depth in cartilage from OA joints (2.3 ± 0.63) than from PM controls (0.56 ± 0.54), ($p = 0.005$). The expression of BSP in patients with OA was associated with the severity of macroscopic cartilage lesions ($r = 0.5$; $p = 0.01$). Highly significant correlations were also observed with the modified Mankin score ($r = 0.71$; $p < 0.0001$) and with the individual scoring criteria of cartilage surface integrity ($r = 0.51$; $p = 0.01$), chondrocyte appearance ($r = 0.44$; $p = 0.03$) and proteoglycan loss ($r = 0.61$; $p = 0.001$).
Conclusions: Genetic and protein expression of BSP are associated with hypertrophic differentiation of chondrocytes in OA. Location of BSP in OA cartilage is clearly associated with macroscopic and microscopic cartilage lesion severity. BSP may be an important factor in cartilage degradation and its role as an angiogenesis enhancer in OA is still to be demonstrated.

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COMMON AND EARLY CARTILAGE DEGENERATION PATTERNS IN AGING AND OSTEOARTHRITIC HUMAN KNEES

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Purpose: A better understanding of early tissue degeneration patterns in the aging knee can help to find new targets for osteoarthritis (OA) prevention and treatment. The objective of this study was to identify common and early morphologic patterns of cartilage degeneration during human joint aging, and to establish their correlation with changes in anterior cruciate ligament (ACL), meniscus, and synovium.
Methods: 130 human knees (67 donors, age range 23–96), obtained from tissue banks, were divided into eight age groups: 21–30 ($n = 8$), 31–40 ($n = 6$), 41–50 ($n = 18$), 51–60 ($n = 21$), 61–70 ($n = 22$), 71–80 ($n = 20$), 81–90 ($n = 26$) and >91 ($n = 9$). All cartilage compartments were macroscopically assessed according to the ICRS mapping system by using a modified Outerbridge grading system. Menisci and ACL were evaluated macroscopically and by histopathology. Synovium histopathology was assessed using a modified Krenn grading system for chronic synovitis. Osteophytosis was macroscopically assessed.
Results: We found a strong correlation between cartilage, meniscus, and ACL degeneration with aging. The earliest cartilage lesions were detected in the central area of the lateral tibial plateau that is not covered by the meniscus, followed by changes in patella and trochlea. Degeneration in the medial femoral condyle appeared later than in the tibia plateau but progressed rapidly to become the most severely affected among all articular surfaces. On the other hand, degeneration in the lateral femoral condyle progressed less rapidly in all age groups. In the lateral TFJ, more knees had a higher tibial than femoral average grade at all ages. This pattern was similar but reversed with aging in the medial TFJ. Degeneration of the meniscus-covered tibia surface correlated with increased meniscus degeneration. A common macroscopic pattern (33%) was degeneration in tibia and meniscus with a normal-appearing femoral condyle. In 14% we found degenerative changes in the tibia while menisci and femoral condyles were normal. In 18% we found a macroscopically normal meniscus while there were degenerative changes in tibia (uncovered portion) and femoral condyle. ACL scores increase with cartilage degeneration at all stages of OA development and correlated with cartilage as well as meniscus degeneration, especially in the medial compartment. Moderate and severe OA groups showed significantly higher ACL substance scores than the minimal/mild OA groups. Synovitis correlated with cartilage degeneration and the presence of osteophytes. In our donor population every knee with osteophytes was accompanied by synovitis.
Conclusions: By examining younger age groups, we were able to identify early patterns of cartilage degeneration. Our data suggest that in younger subjects the lateral tibia plateau shows the earliest and most severe degeneration. Contrary to the notion that the medial TFJ compartment in severe OA is most affected, we found that the lateral tibia plateau is affected the earliest. The uncovered part of the tibia plateau degenerated first, followed by early surface degeneration in the inner rim of the menisci. Degeneration of the covered tibia region increased with cumulative meniscus degeneration, supporting the protective role of menisci. This cross-sectional study emphasizes the importance of interaction of the different joint tissues in OA initiation and progression and defines specific regions in the joint for future studies on biomechanical and cellular factors that determine early stages of OA initiation to reveal novel targets for preventing or delaying OA.

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COMPARISON OF SYNOVIAL FLUID ARGS CONCENTRATIONS AT BASELINE AND ONE-YEAR POST-ACL RECONSTRUCTION COMPARED TO HEALTHY, MATCHED CONTROLS

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Purpose: The clinical signs and symptoms of post-traumatic osteoarthritis (PTOA) following severe joint injury, such as that associated with anterior cruciate ligament (ACL) tears, typically present 10–20 years